Entrez PubMed
Overview
Help | FAQ
Tutorial
New/Noteworthy
E-Utilities

PubMed Services
Journal Browser
MeSH Browser
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
LinkOut
Cubby

Related Resources
Order Documents
NLM Gateway
TOXNET
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

Privacy Policy

1: J Biol Chem 1996 Apr 12;271(15):8618-26

FREE full text article at www.jbc.org

Preparation and characterization of soluble recombinant heterotrimeric complexes of human lymphotoxins alpha and beta.

Browning JL, Miatkowski K, Griffiths DA, Bourdon PR, Hession C, Ambrose CM, Meier W.

Department of Protein Engineering, Biogen, Cambridge, Massachusetts 02142, USA.

The lymphotoxin (LT) protein complex is a heteromer of alpha (LT-alpha, also called tumor necrosis factor (TNF)-beta) and beta (LT-beta) chains anchored to the membrane surface by the transmembrane domain of the LT-beta portion. Both proteins belong to the TNF family of ligands and receptors that regulate aspects of the immune and inflammatory systems. The LT complex is found on activated lymphocytes and binds to the lymphotoxin-beta receptor, which is generally present on nonlymphoid cells. The signaling function of this receptor-ligand pair is not precisely known but is believed to be involved in the development of the peripheral lymphoid organs. To analyze the properties of this complex, a soluble, biologically active form of the surface complex was desired. The LT-beta molecule was engineered into a secreted form and co-expressed with LT-alpha using baculovirus/insect cell technology. By exploiting receptor affinity columns, the LT-alpha3, LT-alpha2/beta1, and LT-alpha1/beta2 forms were purified. All three molecules were trimers, and their biochemical properties are described. The level of LT-alpha3-like components in the LT-alpha1/beta2 preparation was found to be 0.02% by following the activity of the preparation in a WEHI 164 cytotoxicity assay. LT-alpha3 with an asparagine 50 mutation (D50N) cannot bind the TNF receptors. Heteromeric LT complexes were prepared with this mutant LT- alpha form, allowing a precise delineation of the extent of biological activity mediated by the TNF receptors. A LT-alpha3 based cytotoxic activity was used to show that the LT-alpha1/beta2 form cannot readily scramble into a mixture of forms following various treatments and storage periods. This biochemical characterization of the LT heteromeric ligands and the demonstration of their stability provides a solid foundation for both biological studies and an

analysis of the specificity of the LT-bet a and TNF receptors for the various LT forms.

PMID: 8621492 [PubMed - indexed for MEDLINE]

1: J Exp Med 1996 Mar 1;183(3):867-78

Signaling through the lymphotoxin beta receptor induces the death of some adenocarcinoma tumor lines.

Browning JL, Miatkowski K, Sizing I, Griffiths D, Zafari M, Benjamin CD, Meier W, Mackay F.

Department of Immunology and Inflammation, Biogen, Cambridge, Massachusetts 02142, USA.

Surface lymphotoxin (LT) is a heteromeric complex of LT-alpha and LT-beta chains that binds to the LT-beta receptor (LT-beta-R), a member of the tumor necrosis factor (TNF) family of receptors. The biological function of this receptor-ligand system is poorly characterized. Since signaling through other members of this receptor family can induce cell death, e.g., the TNF and Fas receptors, it is important to determine if similar signaling events can be communicated via the LT-beta-R. A soluble form of the surface complex was produced by coexpression of LT-alpha and a converted form of LT-beta wherein the normally type II LT-beta membrane protein was changed to a type I secreted form. Recombinant LT-alpha 1/beta 2 was cytotoxic to the human adenocarcinoma cell lines HT-29, WiDr, MDA-MB-468, and HT-3 when added with the synergizing agent interferon (IFN) gamma. When immobilized on a plastic surface, anti-LT-beta-R monoclonal antibodies (mAbs) induced the death of these cells, demonstrating direct signaling via the LT-beta-R. Anti-LT-beta-R mAbs were also identified that inhibited ligand-induced cell death, whereas others were found to potentiate the activity of the ligand when added in solution. The human WiDr adenocarcinoma line forms solid tumors in immunocompromised mice, and treatment with an anti-LT-beta-R antibody combined with human IFN-gamma arrested tumor growth. The delineation of a biological signaling event mediated by the LT-beta-R opens a window for further studies on its immunological role, and furthermore, activation of the LT-beta-R may have an application in tumor therapy.

PMID: 8642291 [PubMed - indexed for MEDLINE]

1: J Immunol 1995 Jan 1;154(1):33-46

Characterization of surface lymphotoxin forms. Use of specific monoclonal antibodies and soluble receptors.

Browning JL, Dougas I, Ngam-ek A, Bourdon PR, Ehrenfels BN, Miatkowski K, Zafari M, Yampaglia AM, Lawton P, Meier W, et al.

Department of Immunology and Inflammation, Biogen, Cambridge, MA 02142.

Lymphotoxin (LT) is a cytokine related to TNF, found in human systems in both secreted and membrane bound forms. The well characterized secreted form is a trimer of a single protein, LT-alpha, whereas the surface form is composed of a complex between two related molecules, LT-alpha and LT-beta. Because there is a distinct receptor for the complex, the membrane form is believed to signal via events different from those elicited by TNF and secreted LT-alpha. By using a battery of anti-LT-alpha and LT-beta mAbs, it is clear that two LT surface forms exist on the surface of PMA-activated II-23 cells, a human T cell hybridoma. Assuming that these surface forms are trimers, a minor form appears early after induction having an apparent stoichiometry of LT-alpha 2/beta 1 and is recognized by one group of anti-LT-alpha mAbs and the p55-TNF receptor. The second and predominant form has an apparent LT-alpha 1/beta 2 composition and is recognized by a second group of pantrophic anti-LT-alpha mAbs and the LT-beta receptor. Neither of the heteromeric forms nor a putative LT-beta homotrimeric form were found to be secreted. The properties of surface LT on the II-23 cell system were similar to those of the surface LT forms on Chinese hamster ovary cells transfected with both LT-alpha and LT-beta genes and a number of lymphoid tumor lines. These experiments point toward the LT-alpha 1/beta 2 complex as the predominant membrane form of LT on the lymphocyte surface, and this complex is the primary ligand for the LT-beta receptor.

PMID: 7995952 [PubMed - indexed for MEDLINE]